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A Novel DNA Repair Disorder With Thrombocytopenia, Nephrosis, and Features Overlapping Cockayne Syndrome

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We report on four siblings with Cockayne-like syndrome with thrombocytopenia and nephrotic syndrome. The parents were healthy and consanguineous, consistent with an autosomal recessive mode of disease inheritance. UV irradiation of fibroblasts revealed an intermediate sensitivity between normal and standard Cockayne syndrome (CS) control cells. A genome-wide linkage scan conducted using Affymetrix 10K arrays provided exclusion of the known CS genes in the family, and evidence that the disease gene maps to 1p33-p31.1. Thrombocytopenia has not previously been linked with CS, but two patients with CS in association with nephrotic syndrome have previously been documented and the phenotypes are compared with the patients described here. We suggest that this Cockayne-like phenotype with thrombocytopenia and nephrotic syndrome may be a novel DNA repair disorder, and propose that further investigation of other affected families may help identify the causative genetic defect. © 2009 Wiley-Liss, Inc.

Key words: autosomal recessive inheritance; Cockayne syndrome; DNA repair disorders; linkage analysis; nephrotic syndrome; thrombocytopenia; UV irradiation

INTRODUCTION

Cockayne syndrome (CS) is a rare autosomal recessive disorder with variable expression. The phenotype classically becomes evident in early childhood, and patients present with hyperpigmentation, sunken eyes secondary to subcutaneous lipoatrophy, failure to thrive, short stature and microcephaly. Neurological sequelae include progressive ataxia, global developmental delay, sensorineural deafness and tremors [Cockayne, 1936; Nance and Berry, 1992; Lehmann et al., 1993; Tan et al., 2005]. While approximately 10% of patients with CS develop renal pathology, this rarely leads to serious complications [Hirooka et al., 1988; Sato et al., 1988; Reiss et al., 1996]. Renal changes may vary significantly between affected individuals but typically include tubulointerstitial inflammation, interstitial fibrosis and tubular atrophy [Hirooka et al.,

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1988; Funaki et al., 1996^{Q1}—all consistent with nonspecific end-stage renal disease. A small simplified glomerular structure with wrinkling of the basement membrane appears to be specific to Cockayne Syndrome [Funaki et al., 1996]. Thrombocytopenia has not, to our knowledge, been reported previously in association with CS.

A diagnosis of CS is made on clinical features and can be confirmed by UV irradiation of fibroblasts from the patient. CS cells fail to restore normal levels of RNA synthesis following UV irradiation when compared to control fibroblasts [Mayne and Lehmann, 1982]. Mutations in genes CSA and CSB, coding for proteins involved in transcription-coupled repair, are thought to be responsible for the Cockayne phenotype in the majority of cases [Lehmann, 2003]. There is poor genotype/phenotype correlation [Mallery et al., 1998], and identical mutations in CSB may be associated with either CS or xeroderma pigmentosum (XP) - another DNA repair disorder [Lehmann, 2003]. Mutations in genes XPB, XPD, and XPG can result in a mixed clinical phenotype with neurological features of CS and skin abnormalities associated with XP [Lehmann, 2003].

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Here we report on four siblings affected by a Cockayne-like disorder associated with nephrotic syndrome and thrombocytopenia, and an intermediate fibroblast response to UV irradiation.

CLINICAL REPORT

The parents of the four affected children are healthy, Pakistani first cousins (Fig. 1). The two older siblings (III.1 and III.2) were first referred for genetic evaluation aged 6 and 5, respectively. They were noted to have a distinctive phenotype with severe global developmental delay, short stature and similar dysmorphic features: progeroid facies with microcephaly, frontal bossing, sunken eyes, high nasal bridge and large, prominent, low-set ears. There was no history of photosensitivity, poor vision or deafness.

Patient 1

The eldest sibling (III.1, Fig. 1) weighed 2.8 kg (9th centile) at birth. At 6 years, OFC was 47.5 cm, height 93 cm, and weight 13.2 kg; all well below the 0.4th centile. In addition to the dysmorphic features he shared with his sister, he also had keratinized, purple, nodular lesions on his forehead, the exact nature of which could not be identified. Development was slowly progressive with no loss of skills. At presentation, aged 6, he was able to walk up stairs with two feet per step, draw a circle, had good pincer grip and had a few single recognizable words. Previous diagnostic investigations had been inconclusive. He was noted to have a normal karyotype. Computerized tomography (CT) of his brain at the age of 2 revealed “moderately prominent ventricles and sulci” but was otherwise normal with no evidence of basal ganglia calcification. Urine amino acids were normal and congenital infection screen was negative. He had further investigations at the age of 7, during admission to hospital for steroid-unresponsive nephrotic syndrome. A renal biopsy identified glomerular sclerosis, tubular atrophy, interstitial fibrosis, and hyaline thickening of the arteriolar wall, consistent with the non-specific end-stage renal pathology typically observed

in CS. The patient was thrombocytopenic on admission (platelet count: $50 \times 10^9/L$) and had a history of easy bruising. A bone marrow biopsy showed hypocellular particulate bone marrow with representation of all three hematopoietic precursor cell lines including erythrocytes, leucocytes, and thrombocytes. A blood smear was normal except for a few spherocytes, consistent with mild hemolysis. The hematological findings were reviewed and felt to be consistent with a hereditary thrombocytopenia. Normal sensitivity to diepoxybutane excluded Fanconi anemia. Generalized osteoporosis and delayed bone age was apparent on skeletal survey. Recovery of RNA synthesis following UV irradiation of skin fibroblasts gave an intermediate result between normal and known CS controls (Fig. 2). At the age of 8 he died secondary to complications of nephrotic syndrome.

Patient 2

The second child (III.2, Fig. 1) was born at 41 weeks by normal vaginal delivery, weighing 3.1 kg (50th centile). The neonatal period was complicated by hypotonia, and she required 2 days admission to the Special Care Baby Unit. At presentation aged 5, OFC was 44.5 cm and length 87 cm. At age 7 all growth parameters were below the 0.4th centile. Like her brother, this patient had slowly progressive global developmental delay with no loss of skills. She walked at 21 months, and by age 7 she had severe developmental delay particularly affecting speech with only a few single words. A skeletal survey revealed delayed bone age with non-specific changes. Borderline growth hormone response was noted, and the patient was started on replacement therapy at the age of 9. She subsequently developed nodular lesions on her skin similar to those of her elder brother and persistent thrombocytopenia. Normal sister chromatid exchanges for Bloom syndrome, diepoxybutane sensitivity and chromosomal ionizing radiation excluded Bloom syndrome, ataxia telangiectasia and Fanconi anemia, respectively. Attempts to establish a fibroblasts culture for UV sensitivity testing were unsuccessful, and parents declined a second attempt. She developed

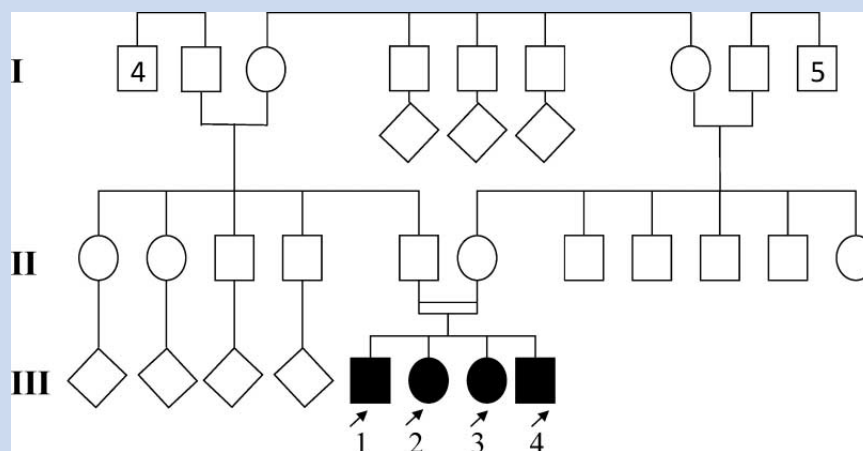


FIG. 1. Pedigree showing the four affected siblings (III.1, III.2, III.3, III.4) as filled black symbols. No other family members are known to be affected and are represented as unfilled symbols. Circles denote females and squares denote males. Individuals of unknown gender are represented by a rhombus.

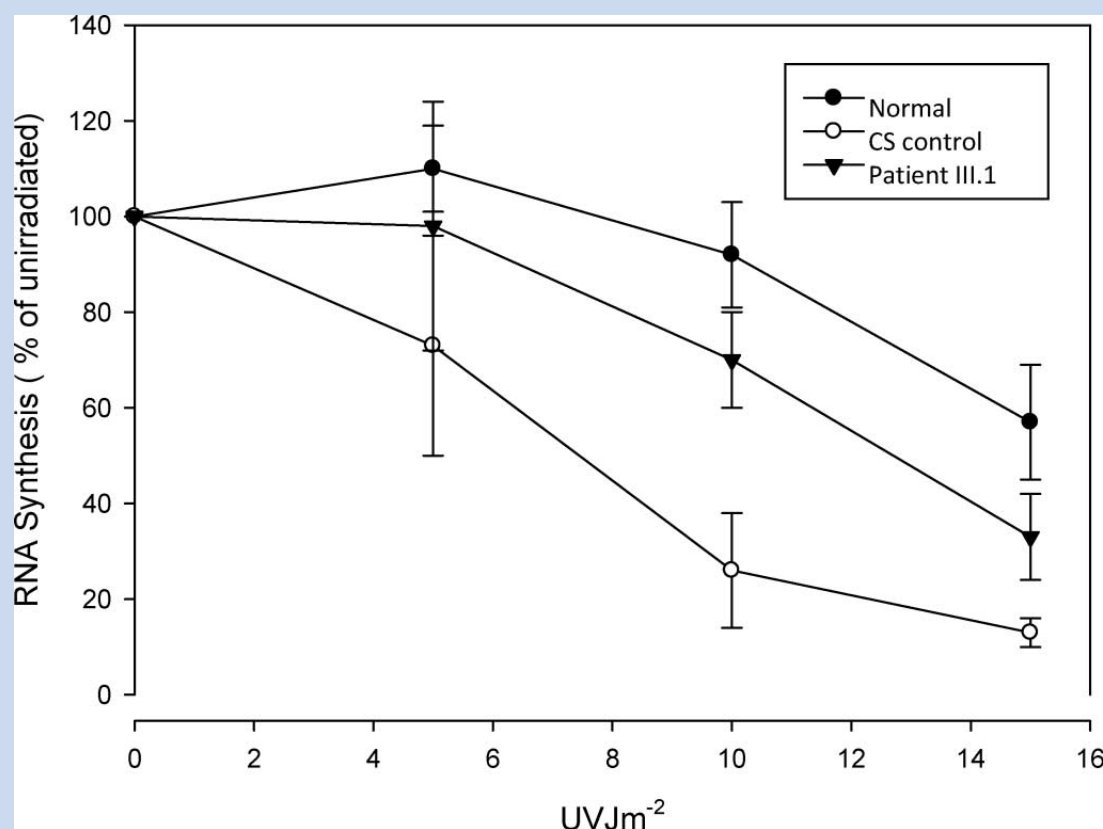


FIG. 2. Recovery of RNA synthesis following UV irradiation of skin fibroblasts from patient III.1. Pooled data from four experiments show an intermediate result between normal and Cockayne syndrome controls.

steroid-responsive nephrotic syndrome aged 10, and died secondary to complications at the age of 16.

Patient 3

The third child (III.3, Fig. 1; Fig. 3a) was born at 42 weeks by spontaneous vaginal delivery with no perinatal complications. Her birth weight was 2.57 kg (0.4th centile) with a head circumference also at the 0.4th centile. The patient was globally delayed from the outset; she was able to sit alone by 1 year, crawled at 18 months, walked at 21 months and had a vocabulary of five or six words by 29 months. Development was assessed at 29 months and although the child was progressing, she had global developmental delay corresponding to a corrected developmental age of 18 months. There was no history of loss of skills. A karyotype was normal. CT brain performed at the age of 2 was normal with no evidence of intracranial calcification. By 14 months she had developed the Cockayne-like facial features of her two older siblings including sunken eyes, frontal bossing, large prominent low set ears, a high nasal bridge and microcephaly, although she had not yet developed any skin papules. Vision and hearing were normal and there was no evidence of photosensitivity. She exhibited gross motor delay, but otherwise appeared less severely affected than her siblings. UV irradiation of fibroblasts again gave a response intermediate

between that of normal and CS cells, similar to her brother. Testing for carbohydrate deficient glycoprotein syndrome revealed normal transferrins. At the age of 6 she has developed persistent moderate thrombocytopenia and proteinuria.

Patient 4

The youngest sibling (III.4, Fig. 1) had an uncomplicated delivery. At birth his weight was 3.2 kg (25th centile) and head circumference below the 9th centile. He sat at 10 months, walked at 18 months and had a vocabulary of one word at 21 months. By the age of 25 months he had mild global developmental delay corresponding to a developmental age of 18 months. There was no history of loss of skills. The patient has similar dysmorphic features to his siblings (Fig. 3b,c) and like his siblings, he has no evidence of photosensitivity, visual problems or deafness. Although he appears to be affected by the same condition as his three siblings he has not yet developed nephrotic syndrome or thrombocytopenia.

LINKAGE ANALYSIS

The parents of these four affected siblings are consanguineous indicating a likely autosomal recessive mode of inheritance. To search for a disease locus a genome wide linkage scan of the parents

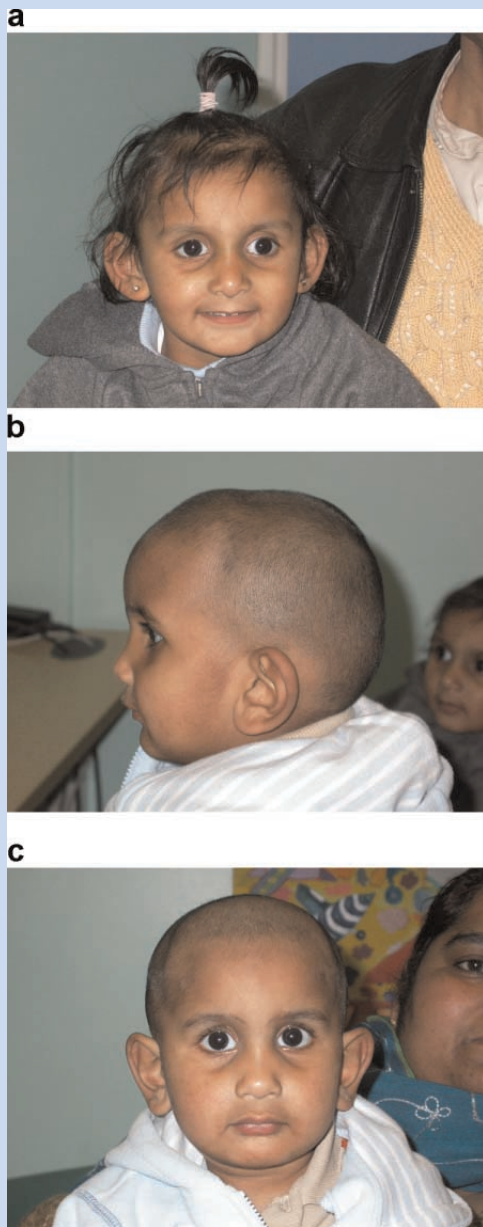


FIG. 3. a: Clinical pictures of Patient 3 at 3 years and 11 months. b,c: Clinical pictures of Patient 4 at 1 year and 6 months. The images demonstrate the phenotype: sunken eyes, frontal bossing, low-set ears and microcephaly. Informed consent was obtained for publication of these pictures. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

and all four siblings was conducted using GeneChip mapping 10K Xba 142 Arrays according to manufacturers protocol (Affymetrix Inc., Santa Clara, CA). Segregation of SNP markers in accordance with affected versus unaffected members of the family excluded the possibility that the disease was caused by dominant or recessive mutations in any of the known CS genes, CSA on chromosome 5, CSB on chromosome 10, XPB on chromosome 2, XPD on chromosome 19 and XPG on chromosome 13.

A 27 Mb interval of shared homozygosity was identified encompassing 104 informative SNPs (defined by dsSNPs rs2354462 to rs718883) at chromosome 1p33–p31.1. Multipoint linkage analysis assuming a fully penetrant autosomal recessive mode of inheritance was undertaken using the GENEHUNTER program [Kruglyak et al., 1995]. Allele frequencies for each marker were assumed to be equal and a population disease gene frequency of 0.001 was used to estimate the maximum LOD score. Statistical support for linkage was evaluated over a range of marker allele frequencies. The map order and distances between markers were based on the UCSC Human Genome Browser (<http://genome.ucsc.edu/>). The multipoint LOD score across the 27 Mb region of linkage was 2.4.

A contiguous gene syndrome cannot be excluded; but any such deletion would have to be <170 kb in size, as genotype signatures were obtained from 104 markers mapping to the region of linkage.

One hundred thirty-one transcripts map to the 27 Mb region of linkage (UCSC Human Genome Browser, March 2006). Excluding the predicted or hypothetical genes mapping to the region that have little or no associated information regarding their biological function, there are no obvious candidate genes at present.

DISCUSSION

The four siblings described in this report have many of the classic features associated with CS. However, some characteristic findings are missing: they all have normal hearing and vision, no evidence of sun-sensitivity or intracranial calcification on brain CT imaging. A literature search did not reveal any other reports of CS in association with thrombocytopenia. Two other children with a diagnosis of CS, who have died as a result of nephrotic syndrome, have previously been described [Reiss et al., 1996; Funaki et al., 2006]. In the report by Reiss et al. [1996], there was a marginal hypersensitivity to sister chromatid exchanges induced by UVC but not to those induced by UVB. The report by Funaki et al. [2006] states that “she underwent a skin biopsy that demonstrated ultraviolet-induced damage,” without any data being presented. It is therefore difficult to assess the UV sensitivity status of these patients’ cells.

It is striking that, in addition to having nephrotic syndrome, the child reported by Reiss et al. [1996] was phenotypically very similar to the children described in this report: he had many classic CS features but did not have the typical photosensitive rash or deafness. Nephrotic syndrome in this child was unresponsive to steroids [Reiss et al., 1996], whereas the patient described by Funaki et al. [2006] was treated with oral prednisolone with good effect. Table I compares the phenotype of the siblings described in this report and the other reported children with CS and nephrotic syndrome.

In this case report Patient 1 had steroid resistant nephrotic syndrome, whilst Patient 2 was responsive to steroid treatment and Patient 3 had persistent proteinuria. This may represent the natural progression of renal pathology in this disorder with initial proteinuria deteriorating into nephrotic syndrome. It may be that the renal pathology seen in this family develops from steroid responsive to steroid unresponsive nephropathy. Alternatively, Patient 2 may have had a partial response to corticosteroid therapy in the early stages of a developing resistant nephrosis.

The authors are aware of about 10 other patients where irradiation of fibroblasts has produced an intermediate response of RNA

TABLE I. Phenotype Comparison Between Classic CS, Children Described in the Literature With CS and Nephrotic Syndrome, and the Patients Described in This Report

	Cockayne syndrome	Patient reported by Reiss et al. [1996]	Patient reported by Funaki et al. [2006]	III.1	III.2	III.3	III.4
Developmental delay	+	+	+	+	+	+	+
Hyperpigmentation	+	+	+	+	+	—	—
Loss of subcutaneous tissue around the eye	+	+	+	+	+	+	+
Microcephaly	+	+	+	+	+	+	+
Small stature	+	+	+	+	+	+	+
Peripheral neuropathy	+	—	+	—	—	—	—
Optic abnormalities	+	—	+	—	—	—	—
Deafness	+	—	—	—	—	—	—
Nephrotic syndrome	—	+	+	+	+	+	—
Sun-sensitive rash	+	—	+	Late onset	Late onset	—	—
Response to UV irradiation	Confirms	Possible increase in sister chromatid exchange	Ambiguous	Intermediate RNA repair	Not done	Intermediate RNA repair	Not done
Age at death	Mean 12.3 (5)	6	12	7	16	Alive	Alive

synthesis recovery following UV irradiation (A.R. Lehmann, unpublished work). Limited information exists regarding phenotype and results of molecular diagnostic testing in these patients. At least three of the patients appear to have a clear Cockayne-like phenotype. However, none are known to have developed nephrotic syndrome.

Collectively these data suggest that the CS-like phenotype we have identified in four affected individuals from a single family represents a distinctive syndrome, possibly a consequence of a novel DNA repair disorder. As the parents of the patients we report were consanguineous, autosomal recessive inheritance is most likely, though other modes of transmission, including mitochondrial inheritance, remain a possibility. On the basis of linkage data it appears that the disease gene maps to 1p. Investigation of additional affected families should, as well as further elucidate this emerging phenotype, facilitate identification of the causal gene.

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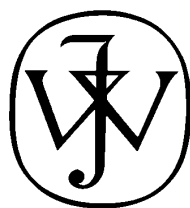
We are grateful to the family for their participation and support of this article.

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